

## BUKTI KORESPONDENSI JURNAL NASIONAL

Judul Artikel : The Power Of Red Shoot Leaf Extract: How Solvent Variations Affect Bone Healing After Tooth Extraction In Rats

Koresponden : Ika Rachmawati

Penulis : Ika Rachmawati, Risyandi Anwar, Megawati Prajarini, Salma Mei Asti, Ita Febriyani

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Our decision is to: Accepted Submission

Nur Khamilatusy Sholekhah  
 Faculty of Dentistry, University Muhammadiyah Semarang  
 Phone 081227384765  
 Fax 02476740241  
[drg.tusy@unimus.ac.id](mailto:drg.tusy@unimus.ac.id)

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Our decision is to: Revision Required

Nur Khamilatussy Sholekhah  
 Faculty of Dentistry, University Muhammadiyah Semarang  
 Phone 081227384765  
 Fax 02476740241  
[drg.tusy@unimus.ac.id](mailto:drg.tusy@unimus.ac.id)

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Reviewer A:  
 i have now completed my review of "THE POWER OF RED SHOOT LEAF EXTRACT: HOW SOLVENT VARIATIONS AFFECT BONE HEALING AFTER TOOTH EXTRACTION IN RATS" for Indonesian Journal of Dentistry, and submitted my recommendation, "Accepted Submission."



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## **LETTER OF ACCEPTANCE**

February, 20<sup>th</sup>, 2025

Dear Author,

On behalf of Indonesian Journal of Dentistry Editorial, we are pleased to inform you that your paper entitled:

**“THE POWER OF RED SHOOT LEAF EXTRACT: HOW SOLVENT VARIATIONS AFFECT BONE HEALING AFTER TOOTH EXTRACTION IN RATS ”**

written by: Ika Rachmawati

has been accepted and will be published in Indonesian Journal of Dentistry Volume 5, Number 1, February 2025

Regards,

drg. Ani Megawati, Sp.PM.

Chief Editor

**Indonesian Journal of Dentistry** has been indexed in:



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Reviewer A:

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#### **INTRODUCTION**

Numerous conditions, including endodontic pathology, periodontitis, face trauma, and forceful extraction techniques, can cause alveolar bone loss. According to the 2018 Basic Health Research, tooth extraction was the second most common procedure performed to treat dental and oral health issues, accounting for 7.9% of the 57.6% of Indonesians with such issues.<sup>1</sup> Complications may arise from a poor tooth extraction. Significant trauma during extraction may cause damage to the tooth's alveolar bone and interfere with the local wound-healing process<sup>2</sup>

Hemostasis, inflammation, proliferation, and remodeling are the stages of the bone remodeling process. A contemporary viewpoint on residual ridge remodeling, starting with the series of inflammatory responses that begin as soon as a tooth is extracted. Blood from the cut vessels, which include proteins and injured cells, fills the socket. Within the first 24 hours, these cells start a chain of events that results in the construction of a fibrin network, which combines with platelets to create a "blood clot" or "coagulum."<sup>3</sup> The coagulum, which functions as a physical matrix, guides the migration of growth factors and cells, including mesenchymal cells. To sterilize the wound, neutrophils and later macrophages enter the area and eliminate bacteria and tissue fragments. In order to accelerate and increase mesenchymal cell migration and their synthetic activity within the coagulum, fibroblasts release growth factors and cytokines.<sup>1</sup>



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Please add some reference why using wistar rat for this research

## THE POWER OF RED SHOOT LEAF EXTRACT: HOW SOLVENT VARIATIONS AFFECT BONE HEALING AFTER TOOTH EXTRACTION IN RATS



### Keywords:

Red shoot leaves, solvent, bone remodeling.

### ABSTRACT

**Background:** A popular medicinal plant in Indonesia, red shoot leaves (*Syzygium myrtifolium* Walp) have been extensively explored for their biological activity and potential to promote hard tissue healing in tooth sockets after extraction. However, few studies have examined this potential. Analyzing the impact of red leaf extract with different solvents on alveolar bone repair in the post-extraction socket in Wistar rats was the aim of this investigation.

**Method:** This study aimed to determine differences in the healing process of bone after tooth extraction. Conducted on 15 male Wistar rats (12 weeks old, weighing 150 – 200 grams). The experiment includes three groups: Group A (5 animals received a gel base as a negative control group), Group B: (5 animals received 2% n-hexane gel treatment of red shoot leaves) and Group C (5 animals received 2% extract ethanol gel of red shoot leaves, based on designated observation days 30. The assessment involves bone remodeling area o from radiograph.

**Outcome:** The average alveolar bone density varies greatly, as indicated by the study's p value of 0.000 ( $p < 0.005$ ). The group that had 2% n-hexane gel treatment for red shoot leaves had the highest average alveolar bone density. According to the results of the phytochemical screening test, the n-hexane extract included triterpenoid and steroids, but the ethanol extract contained flavonoids, tannins, and alkaloids. The findings of this study demonstrate that the use of solvents with varying polarity can have an impact on bone remodeling.

**Conclusion:** The alveolar bone mineral density of Wistar rats following tooth extraction was increased by 2% n-hexane gel treatment of red shoot leaves.

## INTRODUCTION

Numerous conditions, including endodontic pathology, periodontitis, face trauma, and forceful extraction techniques, can cause alveolar bone loss. According to the 2018 Basic Health Research, tooth extraction was the second most common procedure performed to treat dental and oral health issues, accounting for 7.9% of the 57.6% of Indonesians with such issues.<sup>1</sup> Complications may arise from a poor tooth extraction. Significant trauma during extraction may cause damage to the tooth's alveolar bone and interfere with the local wound-healing process<sup>2</sup>

Hemostasis, inflammation, proliferation, and remodeling are the stages of the bone remodeling process. A contemporary viewpoint on residual ridge remodeling, starting with the series of inflammatory responses that begin as soon as a tooth is extracted. Blood from the cut vessels, which include proteins and injured cells, fills the socket. Within the first 24 hours, these cells start a chain of events that results in the construction of a fibrin network, which combines with platelets to create a "blood clot" or "coagulum."<sup>3</sup> The coagulum, which functions as a physical matrix, guides the migration of growth factors and cells, including mesenchymal cells. To sterilize the wound, neutrophils and later macrophages enter the area and eliminate bacteria and tissue fragments. In order to accelerate and increase mesenchymal cell migration and their synthetic activity within the coagulum, fibroblasts release growth factors and cytokines.<sup>1</sup>

The blood clot breaks down (fibrinolysis) in a few of days. Granulation tissue gradually replaces the coagulum as a result of mesenchymal cell proliferation (2–4 days).<sup>4</sup> A vascular network forms after one week, and by two weeks, young connective tissue that is abundant in inflammatory cells and arteries has covered the edge of the extraction socket. By four to six weeks, the soft tissue of the alveolus has keratinized and the majority of it is filled with woven bone. Layers of lamellar bone are produced on top of the previously developed woven bone around 4–6 months to fortify the mineral tissue within the initial socket.<sup>5</sup> The soft tissue of the alveolus gets keratinized by 4–6 weeks, and the majority of the alveolus is filled with woven bone. Between four and six months, layers of lamellar bone are deposited on top of the previously created woven bone to fortify the mineral tissue inside the original socket.<sup>3</sup> For several months, bone will continue to deposit in the socket, but it won't reach the neighboring teeth's coronal bone level.<sup>2</sup>

New vascularized tissue is a key component that aids in the quick healing of injured wounds, including bone-related ones. Among other things, the angiogenesis process initiates bone vascularization. Basic fibroblast growth factor (bFGF) is one of the elements that significantly contributes to bone vascularization. Fibroblasts contribute to the formation of blood vessel walls via means of bFGF. The differentiation of chondrocytes and osteoblasts that emerge in the early stages of proliferation during the bone healing process and the development of vascularization in new bone are mediated by bFGF.<sup>3,5,6</sup> bFGF is a growth factor that aids in tissue formation and bone remodeling and can trigger phases of the angiogenesis process.<sup>1,4,7</sup> bFGF is regarded as a growth factor in tissue repair that contributes to the promotion of endothelial migration, proliferation, and blood vessel creation. To stimulate the proliferation of new tissue, bFGF bonds with platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF).<sup>8</sup>

It may be necessary to create new blood vessels since the extraction procedure may damage some of the blood vessels in the tooth socket. Another criterion for the effectiveness of the bone

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remodeling process is the development of new blood vessels. The development of new blood vessels is crucial to the bone remodeling process because they can replace damaged ones and provide nourishment to newly created cells in the wound tissue.<sup>12</sup> Furthermore, the wound cannot heal appropriately if it fails during one of the healing phases following tooth extraction, particularly the angiogenesis process.<sup>3</sup>

Following tooth extraction, medications can lessen problems and hasten the healing of the wound. Complications may arise from inappropriate tooth extraction. According to several researchers, it requires anti-inflammatory medications.<sup>9</sup> In addition to oral consumption, herbal preparations are useful for improving bone healing when applied locally. Of particular interest are phytochemical (polyphenol) substances, which are a particular novel drug period. In order to satisfy the criteria of the regenerative process, numerous researchers were interested in examining the drying method, appropriate dosage, solvent extraction, and local application delivery systems. As a result, there isn't a single extraction technique that works for all plant phenols. The local application of phenolic compounds in clinical practice will soon be a reality. Polyphenols are thought to work by inhibiting osteoclast development and function. Additionally, to promote osteoblast growth while lowering the rate of apoptosis.<sup>10</sup>

Due to their low side effects, herbal medications are increasingly being used in place of chemical ones. Red shoot leaf (*Syzygium Myrt folium Walp*) was one of the plants. Triterpenoid and steroid alkaloids are among the active compounds found in this plant. By reducing wound swelling that happens during the wound inflammation healing phase, steroid chemicals offer anti-inflammatory qualities.<sup>11</sup> Alkaloid chemicals' antibacterial and anti-inflammatory qualities aid in the first wound's blood vasoconstriction process, reducing bleeding during the stages of inflammation and proliferation. A triterpenoid molecule called botulinic acid (3 $\beta$ -3-Hydroxy-lup-20(29)-en-28-oic acid) is present in red shoot leaves. By inhibiting the role of osteoclast resorption in the bone-healing process, botulinic acid has the ability to decrease the hydroxyapatite resorption process. Bone density will be impacted by hydroxyapatite. Additionally, this study sought to examine how red leaf extract with different solvents affected the healing of alveolar bone in the Wistar rats' post-extraction socket.<sup>8,12</sup>

## RESEARCH METHODS

The Ethics Committee of the Universitas Muhammadiyah Semarang, Faculty of Nursing and Health Sciences has accepted this study with letter number No. 100/KE/02/2024. This kind of study used a post-test-only control group design in a lab setting. The UNPAD laboratory was used to prepare an n-hexane extract of red shoot leaves and conduct phytochemical studies. In the STIFAR lab, red shoot leaf n-hexane extract gel was manufactured. In the animal laboratory at UNIMUS, maintenance, treatment, and control were performed. At the Griya Satwa Lestari Semarang veterinary clinic, radiographs were taken.



This study used the maceration method to create the extract. The dried red shoot leaf *simplicia*, weighing 1 kg, was then steeped for 24 hours at room temperature in 30 liters of 96% ethanol. Moreover, the pulp and filtrate were separated using a Buchner funnel. Five times, the macerate was collected, shaking occasionally. A rotary evaporator set to 40°C was used to concentrate the final filtrate, yielding 310.55 grams of pure extract.

This study employed the separating funnel method for the n-hexane fractionation of red shoot leaves. Ten grams of concentrated extract are added at the start of the procedure, dissolved in 65 ml of water and 10 ml of ethanol. Put it in a separating funnel and use liquid-liquid extraction to add 75 ml of n-hexane until the n-hexane fraction is clear. Re-concentrate using a rotary evaporator set to 40°C after separating the n-hexane fraction (top layer) from the water fraction (bottom layer).

The phytochemical analysis of n-hexane red shoot leaves reveals the presence of steroids, triterpenoid, and alkaloids, dedangkan ekstrak ethanol daun pucuk merah terdiri dari kandungan flavonoid, alkaloid dan tanin. This study used 3% Na-CMC as a gelling agent to create the n-hexane extract of red shoot leaves and extract ethanol of red shoot leaves. The procedure started with a mild addition of 3% Na-CMC to 10 ml of hot water, followed by a 15-minute wait until the color changed to a clear gel. After that, distilled water was used to dilute the homogenous mass by 100 ml. The process of creating a concentrated extract gel started with adding the extract, dissolving it with a little amount of water, and heating it to 50°C. Next, 3 grams of Na-CMC were added, and the mixture was stirred until it reached a uniform consistency. Glycerin was added up to 100g along with propylene glycol and distilled water, and the mixture was constantly stirred until a semi-solid gel was created. The preparation formula for the n-hexane extract gel and ethanol extract was varied with a concentration of 2%.

Viscosity, spread ability, adhesiveness, pH, and organoleptic tests were used to test the n-hexane extract gel and the ethanol extract of red shoot leaves. The extract gel's color, odor, and form are examined in an organoleptic test. A pH meter is used to determine the pH after 0.5g of gel extract has been dissolved in 50ml of distilled water; a good pH is between 4.5 and 6.5. The purpose of the pH test is to ascertain how stable the gel formulation is over time. To feel comfortable when applied, the pH level needs to be within the typical range; an alkaline pH might result in scaly skin, while an acidic pH can irritate skin.

To measure viscosity, a Brookfield viscometer was used. Following a day of storage, the gel's viscosity was measured. Good gel formulations have viscosity values between 6,000 and 50,000 cps. To perform the spread ability test, 0.5 g of the gel sample was added to a 15 cm-diameter round glass. The gel spread diameter was measured after another glass was set on top of it and allowed to sit for a minute. The constant diameter was measured after adding 150 g of load and letting it stand for one minute. A pleasant consistency for topical application is indicated by a spread ability of 5-7 cm.

The adhesion test involves applying 0.5 grams of gel to a glass plate, then loading it with 300 grams for five minutes. While keeping track of how long it takes for the two plates to separate, the weight is raised off the glass plate and released. A minimum of 4 seconds is required for

optimum adherence.<sup>17</sup> In order for the active ingredient in the gel preparation to be absorbed, the adhesion test is used to measure how long the gel sticks to the contact surface after application.

The population of this research was male Wistar rats (*Rattus norvegicus*), 12 weeks old, with a body weight of 150 – 200 grams. The samples utilized in this study were determined using Ferderer's formula, with a 10% correction applied if there were instances of experimental animal mortality during the study period. As a result, the total sample size for this study comprised 15 mice. Prior to the research, the mice underwent a two week adaptation period, during which their food, drink and maintenance adhered to hospital standards. Additionally, they received worm medicine and vitamins. The extraction procedure commenced with the weighing of all rats, were anesthetized with doses adjusted based on their body weight. Tooth extraction was performed on the left first molar.

The experiment includes three groups: Group A received gel base as a negative control (5 rats), Group B received 2% n-hexane gel treatment of red shoot leaves (5 rats), and Group C received 2% ethanol extract gel treatment of red shoot leaves (5 rats). For 30 days, each rat in each treatment group received 0.1 cc of gel once day using cotton pellets. The rats were put to death on the 30<sup>th</sup> day by the over-ether inhalation method, which involved putting cotton in a jar at the bottom and moistening it with 5 ml of ether. The mice were then left for 15 to 30 minutes until their respiration and pulse could no longer be felt. With all of their legs secured with needles, place them on their backs on a stand. Until the mandibular bone remained intact, the mandibular skin was cleansed. Using blade number 11, the mandibular bone was cut from the right temporomandibular joint to the left temporomandibular joint. The cut was then repeated until only the left mandible remained. After the cut was made, the mandibular bone was cleaned with NaCl until no blood remained. The assessment involves bone remodeling area from radiograph.

A portable veterinary dental X-ray radiography system, the D-X2V, was then used to take pictures of the preserved alveolar bone samples. The radiographic analysis that is calculated is the area of the defect area after tooth extraction on the radiograph. Measurements were carried out using rearrangement measurements on ImageJ software. The procedure involves selecting the radiograph for measurement, zooming in until the image is clear, initiating the measurement with the rectangular feature, recording measurement, and generating the results.









**Image 1.** Mandibular Fixation(A), Mandibular Exposure (B). Bone Density Analyzed (C)

The SPSS 25 statistical data processing software was used to statistically evaluate the results, which included a Shapiro-Wilk test for normality and an evaluation of bone mineral density. Bone mineral density data that has been normal is then subjected to a homogeneity test, which uses the Levene test to homogenize data between groups with a 95% significance level ( $p \geq 0.05$ ). The One-Way Anova parametric test can be used to determine the bone mineral density between groups in data that is normally distributed and homogeneous. After the Anova data revealed a significant difference ( $p < 0.05$ ), the data analysis was carried out to determine the influential group using the Multiple Comparisons Test type LSD (Least Significant Different).



### RESEARCH FINDINGS



The results of the Phytochemical test in table 1. show that the n-hexane extract of red shoot leaves contains steroid and triterpenoid compounds, but the results are negative for alkaloid compounds and in table. 2 shows that the ethanol extract of red shoot leaves contains flavonoid, alkaloid and tannin compounds.

**Table 1.** Results of phytochemical tests of n-hexane on red shoot leaves

Secondary Metabolite Compounds	Test Method	Before	After	test results
Alkaloid	Chlorophom ammoniacal + H <sub>2</sub> SO <sub>4</sub> 2N + Dragendorff reagent			(-) no orange brown color
Triterpenoid	Reagent H <sub>2</sub> SO <sub>4</sub> concentrated 2 drops + CH <sub>3</sub> COOH (Acetic acid) Anhydrous 1 drop			(+) dark brown color
Steroid	Reagent H <sub>2</sub> SO <sub>4</sub> concentrated 2 drops + CH <sub>3</sub> COOH (Acetic acid) Anhydrous 1 drop			(+) bluish green color

**Table 2.** Results of Phytochemical Tests of Ethanol Extract of Red Shoot Leaves

Secondary Metabolite Compounds	Test Method	Before	After	test results
Flavonoid	<i>Chlorophom ammoniacal + Mg powder + alcohol:HCl + amyl alcohol</i>			(+) red color

Alkaloid	Chloroform ammonia White precipitate + H <sub>2</sub> SO <sub>4</sub> 2N + (+) Dragendorff's reagent		(+) white sediment
Tanin	FeCl <sub>3</sub> 1% 2 drops		(+) blackish green color

This study was conducted in three groups, each group was observed after 30 days. The distribution of the data was initially measured, which revealed that the average of the 3 treatment groups of data showed a normal distribution ( $p > 0.05$ ) Table 4.

**Table 3.** Description of data from groups A, B and C.

	Mean	Std. Deviation	Std. Error
Control (-) Group (A)	113.7980	3.94815	1.76567
N-Heksan 2% (B)	149.6800	3.83693	1.71593
Ethanol 2% (C)	129.6400	4.09182	1.82992
Total	131.0393	15.63351	4.03655

**Table 4.** Tests of Normality.

	Statistic	Shapiro-Wilk		Sig.
		df		
Control (-) Group (A)	.920	5		.531
N-Heksan 2% (B)	.933	5		.616
Ethanol 2% (C)	.967	5		.854

**Table 5.** Levene's Test for Homogeneity of variance Result

<i>Levene Test</i>	<b>Signifikansi</b>	<b>Probabilitas</b>
	0.999	P > 0.05

The homogeneity test showed  $p > 0.05$ , suggesting that the data variances were homogeneous. Consequently, a statistical test was performed using the One-Way ANOVA test to determine if there were significant differences among the groups.

**Table 6.** One-Way ANOVA Test Results

<i>One-way Anova</i>	<b>Significance</b>	<b>Probability</b>
	0.000	P < 0.05

From the table 6, it is known that the significance value shows a value of less than 0.05, so it can be concluded that there is a significant difference between the Control, 2% N-Hexane and 2% Ethanol. Subsequently, a post hoc test was conducted to determine the differences between groups.

**Table 7.** post-hoc multiple comparisons (LSD) Result

(I) Group	(J) Group	Mean Difference		
		(I-J)	Std. Error	Sig.
Control (-)	N-Heksan 2%	-35.88200*	2.50474	.000
	Ethanol 2%	-15.84200*	2.50474	.000
N-Heksan 2%	Control (-)	35.88200*	2.50474	.000
	Ethanol 2%	20.04000*	2.50474	.000
Ethanol 2%	Control (-)	15.84200*	2.50474	.000
	N-Heksan 2%	-20.04000*	2.50474	.000

## DISCUSSION

Following tooth extraction, there are multiple stages in the biological process of bone remodeling, including hemostasis, inflammation, proliferation, and remodeling. Granulation tissue will take its place in the socket wound once the bleeding has stopped. The proliferative phase of the bone remodeling process starts when granulation tissue is progressively replaced by a transient connective tissue matrix that is high in collagen fibers.<sup>3</sup> Additionally, collagen is the main protein present in the bone matrix. COL1 is the most Furthermore, the primary protein found in the bone matrix is collagen. The most prevalent kind of collagen protein, COL1, is employed extensively as a bone-preserving agent to promote tissue regeneration in broken bone. Growth factors released by macrophages toward the end of the inflammatory phase trigger the activation of osteoblast cells. By producing proteins that produce new bone, osteoblast cells serve as indicators of alveolar bone healing. These osteoblast cells proceed into the bone remodeling phase after first emerging during the proliferative phase. Apoptosis or osteocyte cell differentiation are two possible outcomes of the maturation process that osteoblast cells go through. Since osteoblasts and osteocytes are indicators of osteogenesis, the number of these cells was calculated. Collagen thickness was determined since it is a measure of the bone remodeling process, and osteoblasts and osteocytes must function in harmony to remodel during osteogenesis.<sup>13</sup>

In our study, 2% n-hexane extract gel of red shoot leaves had a higher effect on increasing the alveolar bone mineral density of Wistar rats after tooth extraction. The results of the data in table 6 show that there is a significant difference between the treatment groups. The results (Table 7) show that there is a difference in bone density, this is influenced by the difference in the material and concentration of red shoot leaf solvent. The results of bone remodeling after tooth extraction using n-hexane gel of red shoot leaves 2% are more significant, because n-hexane is a non-polar solvent, so it only extracts compounds with low polarity. The results of bone density evaluation

using Image-J software revealed that the deposition of newly formed bone trabeculae started from the socket border, growing through the empty socket.

Bone marrow gaps separated the thick, regularly distributed bone trabeculae. With a few more developed lamellar bone appearances, the freshly created bone is mostly composed of woven bone. At five weeks, the newly bone had nearly filled the socket space. Demonstrates that the density rises in the experimental groups, particularly on day 30. After day 28, the medullary canal and trabeculae appear thickened due to the growth of lamellar bone fibers in the socket area. The thickening of trabeculae in this study is in line with bone healing after tooth extraction. Furthermore, this research shows a process soft bone formation that begins in the 4<sup>th</sup> week, as evidenced by a change in the radiographic image from radiolucent to radio intermediate and radiopaque.<sup>1,7</sup>

The findings of the phytochemical analysis of the red shoot leaf n-hexane extract revealed triterpenoid and steroid compounds, which is contrary to earlier research that found secondary metabolites of alkaloid, steroid, and triterpenoid compounds in the n-hexane fraction of red shoot leaves.<sup>14</sup> The phytochemical test in this study did not identify any alkaloids, which may be because ethanol was employed as the solvent for the maceration process and n-hexane was utilized for the fractionation process. Only polar solvents, like ethanol, can dissolve polar molecules, and only non-polar solvents, like n-hexane, will dissolve non-polar compounds. Although alkaloids are typically found in polar solvents, the dissolving process does not take place when n-hexane, a non-polar solvent, is used in the fractionation process. Since steroids are non-polar substances, they can be found using n-hexane solvent during the fractionation process. By blocking the phospholipase enzyme via the arachidonic acid pathway, steroids can contribute to the inflammatory phase.<sup>9,13,15</sup> It is arachidonic acid that forms prostaglandins. When there is cell injury, prostaglandins are released. The enzymes prostaglandin G and prostaglandin H synthetase, sometimes referred to as cyclooxygenase (COX), mediate the first production of prostaglandins. Arachidonic acid is converted by this enzyme into prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), which is then converted into prostacyclin (PGI<sub>2</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), and thromboxane A<sub>2</sub> (TXA<sub>2</sub>). The eventual outcome will be the appearance of inflammation-related symptoms. By boosting the size and quantity of osteoclasts and inducing the activation of preexisting osteoclasts, prostaglandins actually cause bone resorption. The synthesis of prostaglandins will be inhibited by steroids.<sup>15</sup>

Additionally, by preventing the creation of OLCs (Osteoclast-Like cells), triterpenoids can protect bone density by preventing the formation of RANKL, which prevents RANKL from binding RANK and non-induction of osteoclasts. It has been demonstrated that triterpenoids reduce the expression of genes unique to osteoclasts (such as tartrate-resistance acid phosphatase) during the osteoclast genesis process, which in turn reduces osteoclast differentiation. Red shoot leaves are the natural source of botulinic acid, a pentacyclic triterpenoid molecule with numerous pharmacological and biological characteristics, including anti-inflammatory effects. By decreasing Akt and IκB phosphorylation and PLCγ<sub>2</sub>-Ca<sup>2+</sup> signaling in pathways involved in early osteoclasts genesis and subsequently suppressing c-Fos and NFATc1, botulinic acid effectively inhibits osteoclastogenesis.<sup>16</sup> Numerous biological molecules, such as macrophage/monocyte colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANK & RANKL), control osteoclast genesis. When RANKL binds to its receptor RAN, it initiates the first osteoclastogenic signaling. In addition to activating the NF-κB, Akt, and mitogen-activated protein kinase (MAPK) signaling pathways, which include p38, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and calcium ion [Ca<sup>2+</sup>] signaled by phospholipase

C $\gamma$  (PLC $\gamma$ ), the RANK-RANKL complex starts the recruitment of tumor necrosis factor receptor-associated factor 6 (TRAF6). When these pathways are engaged, important transcription factors like nuclear factor of activated T cells c1 (NFATc1) and the Fos proto-oncogene (c-Fos) are induced, which raises the expression of genes unique to osteoclasts.<sup>17</sup>

However, the assessment of bone remodeling after tooth extraction seems to be subject to some limitations. In this research based on the previous investigation, because it considers the time and cost factors. In this study, animal radiography modality was used to assess the bone remodeling process after tooth extraction. This study has limitations, namely that it cannot analyze the increase in the number of trabeculae because changes in the digitalization process reduce quality. For clinical decision making and to complete this study, histopathological analysis is recommended.

## CONCLUSION

Based on the research results, it can be concluded that the type of solvent used in extracting red shoot leaves shows a significant difference in the bone remodeling process. The fastest bone remodeling is found at a concentration of 2% n – hexane.

## ACKNOWLEDGMENTS

We would like to express our sincere gratitude to the Faculty of Dentistry Universitas Muhammadiyah Semarang (FKG Unimus), for their invaluable support and guidance throughout this research.

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## THE POWER OF RED SHOOT LEAF EXTRACT: HOW SOLVENT VARIATIONS AFFECT BONE HEALING AFTER TOOTH EXTRACTION IN RATS

Ika Rachmawati<sup>1</sup>, Risyandi Anwar<sup>2</sup>, Megawati Prajarini<sup>3</sup>, Salma Mei Asti<sup>4</sup>, Ita Febriyani<sup>5</sup>

1 Faculty of Dentistry, Universitas Muhammadiyah Semarang

2 Faculty of Dentistry, Universitas Muhammadiyah Semarang

3. Faculty of Dentistry, Universitas Muhammadiyah Semarang

4. Student Faculty of Dentistry, Universitas Muhammadiyah Semarang

5. Student Faculty of Dentistry, Universitas Muhammadiyah Semarang

### Keywords:

Red shoot leaves, solvent, bone remodeling.

### ABSTRACT

**Background:** A popular medicinal plant in Indonesia, red shoot leaves (*Syzygium myrtifolium Walp*) have been extensively explored for their biological activity and potential to promote hard tissue healing in tooth sockets after extraction. However, few studies have examined this potential. Analyzing the impact of red leaf extract with different solvents on alveolar bone repair in the post-extraction socket in Wistar rats was the aim of this investigation.

**Method:** This study aimed to determine differences in the healing process of bone after tooth extraction. Conducted on 15 male Wistar rats (12 weeks old, weighing 150 – 200 grams). The experiment includes three groups: Group A (5 animals received a gel base as a negative control group), Group B: (5 animals received 2% n-hexane gel treatment of red shoot leaves) and Group C (5 animals received 2% extract ethanol gel of red shoot leaves, based on designated observation after 30 days. After taking a radiograph of the mandible, Image-J software was used to measure the bone remodeling of the extraction socket and the surrounding alveolar bone.

**Outcome:** The average alveolar bone density varies greatly, as indicated by the study's p value of 0.000 ( $p < 0.005$ ). The group that had 2% n-hexane gel treatment for red shoot leaves had the highest average alveolar bone density. According to the results of the phytochemical screening test, the n-hexane extract included triterpenoid and steroids, but the ethanol extract contained flavonoids, tannins, and alkaloids. The findings of this study demonstrate that the use of solvents with varying polarity can have an impact on bone remodeling.

**Conclusion:** The alveolar bone mineral density of Wistar rats following tooth extraction was increased by 2% n-hexane gel treatment of red shoot leaves.

## INTRODUCTION

Numerous conditions, including endodontic pathology, periodontitis, face trauma, and forceful extraction techniques, can cause alveolar bone loss. According to the 2018 Basic Health Research, tooth extraction was the second most common procedure performed to treat dental and oral health issues, accounting for 7.9% of the 57.6% of Indonesians with such issues.<sup>1</sup> Complications may arise from a poor tooth extraction. Significant trauma during extraction may cause damage to the tooth's alveolar bone and interfere with the local wound-healing process<sup>2</sup>

8 Hemostasis, inflammation, proliferation, and remodeling are the stages of the bone remodeling process. A contemporary viewpoint on residual ridge remodeling, starting with the series of inflammatory responses that begin as soon as a tooth is extracted. Blood from the cut vessels, which include proteins and injured cells, fills the socket. Within the first 24 hours, these cells start a chain of events that results in the construction of a fibrin network, which combines with platelets to create a "blood clot" or "coagulum."<sup>3</sup> The coagulum, which functions as a physical matrix, guides the migration of growth factors and cells, including mesenchymal cells. To sterilize the wound, neutrophils and later macrophages enter the area and eliminate bacteria and tissue fragments. In order to accelerate and increase mesenchymal cell migration and their synthetic activity within the coagulum, fibroblasts release growth factors and cytokines.<sup>1</sup>

15 21 The blood clot breaks down (fibrinolysis) in a few of days. Granulation tissue gradually replaces the coagulum as a result of mesenchymal cell proliferation (2–4 days).<sup>4</sup> A vascular network forms after one week, and by two weeks, young connective tissue that is abundant in inflammatory cells and arteries has covered the edge of the extraction socket. By four to six weeks, the soft tissue of the alveolus has keratinized and the majority of it is filled with woven bone. Layers of lamellar bone are produced on top of the previously developed woven bone around 4-6 months to fortify the mineral tissue within the initial socket.<sup>5</sup> The soft tissue of the alveolus gets keratinized by 4–6 weeks, and the majority of the alveolus is filled with woven bone. Between four and six months, layers of lamellar bone are deposited on top of the previously created woven bone to fortify the mineral tissue inside the original socket.<sup>3</sup> For several months, bone will continue to deposit in the socket, but it won't reach the neighboring teeth's coronal bone level.<sup>2</sup>

1 20 New vascularized tissue is a key component that aids in the quick healing of injured wounds, including bone-related ones. Among other things, the angiogenesis process initiates bone vascularization. Basic fibroblast growth factor (bFGF) is one of the elements that significantly contributes to bone vascularization. Fibroblasts contribute to the formation of blood vessel walls via means of bFGF. The differentiation of chondrocytes and osteoblasts that emerge in the early stages of proliferation during the bone healing process and the development of vascularization in new bone are mediated by bFGF.<sup>3,5,6</sup> bFGF is a growth factor that aids in tissue formation and bone remodeling and can trigger phases of the angiogenesis process.<sup>1,4,7</sup> bFGF is regarded as a growth factor in tissue repair that contributes to the promotion of endothelial migration, proliferation, and blood vessel creation. To stimulate the proliferation of new tissue, bFGF bonds with platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF).<sup>8</sup>

30 It may be necessary to create new blood vessels since the extraction procedure may damage some of the blood vessels in the tooth socket. Another criterion for the effectiveness of the bone

14 remodeling process is the development of new blood vessels. The development of new blood vessels is crucial to the bone remodeling process because they can replace damaged ones and provide nourishment to newly created cells in the wound tissue.<sup>12</sup> Furthermore, the wound cannot heal appropriately if it fails during one of the healing phases following tooth extraction, particularly the angiogenesis process.<sup>3</sup>

A popular strain of laboratory rats, Wistar rats (*Rattus norvegicus*) are essential models in biomedical research because of their well-established genetic, physiological, and behavioral traits. Since their invention in the 1900s, Wistar rats have grown to be among the most widely utilized species in scientific research, especially in disciplines like genetics, pharmacology, toxicology, and neuroscience. Rats are ideal subjects for study subjects due to their well-established responses to a variety of experimental methods, easy breeding, and calm action.<sup>9</sup>

10 Following tooth extraction, medications can lessen problems and fasten the healing of the wound. Complications may arise from inappropriate tooth extraction. According to several researchers, it requires anti-inflammatory medications.<sup>10</sup> In addition to oral consumption, herbal preparations are useful for improving bone healing when applied locally. Of particular interest are phytochemical (polyphenol) substances, which are a particular novel drug period. In order to satisfy the criteria of the regenerative process, numerous researchers were interested in examining the drying method, appropriate dosage, solvent extraction, and local application delivery systems. 10 As a result, there isn't a single extraction technique that works for all plant phenols. The local application of phenolic compounds in clinical practice will soon be a reality. Polyphenols are thought to work by inhibiting osteoclast development and function. Additionally, to promote osteoblast growth while lowering the rate of apoptosis.<sup>11</sup>

17 Due to their low side effects, herbal medications are increasingly being used in place of chemical ones. Red shoot leaf (*Syzygium myrtifolium Walp*) was one of the plants. Triterpenoid steroid and alkaloids are among the active compounds found in this plant. By reducing wound swelling that happens during the wound inflammation healing phase, steroid chemicals offer anti-inflammatory qualities.<sup>12</sup> Alkaloid chemicals' antibacterial and anti-inflammatory qualities aid in the first wound's blood vasoconstriction process, reducing bleeding during the stages of inflammation and proliferation. A triterpenoid molecule called botulinic acid (3 $\beta$ -3-Hydroxy-lup-20(29)-en-28-oic acid) is present in red shoot leaves. By inhibiting the role of osteoclast resorption in the bone-healing process, botulinic acid has the ability to decrease the hydroxyapatite resorption process. Bone density will be impacted by hydroxyapatite. Additionally, this study sought to examine how red leaf extract with different solvents affected the healing of alveolar bone in the Wistar rats' post-extraction socket.<sup>8,13</sup>

## RESEARCH METHODS

29 The Ethics Committee of the Universitas Muhammadiyah Semarang, Faculty of Nursing and Health Sciences has accepted this study with letter number No. 100/KE/02/2024. This kind of study used a post-test-only control group design in a lab setting. The UNPAD laboratory was used to prepare an n-hexane and ethanol extract of red shoot leaves, and conduct phytochemical studies. 2 4



In the STIFAR lab, red shoot leaf n-hexane and ethanol extract gel was manufactured. In the animal laboratory at UNIMUS, maintenance, treatment, and control were performed. At the Griya Satwa Lestari Semarang veterinary clinic, radiographs were taken.

4 The phytochemical analysis of n-hexane red shoot leaves reveals the presence of steroids, triterpenoid, and alkaloids, while the ethanol extract of red shoot leaves consists of flavonoids, alkaloids and tannins. This study used 3% Na-CMC as a gelling agent to create the n-hexane extract of red shoot leaves and extract ethanol of red shoot leaves. The preparation formula for the n-hexane extract gel and ethanol extract was varied with a concentration of 2%.

4 Viscosity, spread ability, adhesiveness, pH, and organoleptic tests were used to test the n-hexane extract gel and the ethanol extract of red shoot leaves. The extract gel's color, odor, and form are examined in an organoleptic test. The purpose of the pH test is to ascertain how stable the gel formulation is over time. To feel comfortable when applied, the pH level needs to be within the typical range; an alkaline pH might result in scaly skin, while an acidic pH can irritate skin.

4 To measure viscosity, a Brookfield viscometer was used. Following a day of storage, the gel's viscosity was measured. Good gel formulations have viscosity values between 6,000 and 50,000 cps. To perform the spread ability test, 0.5 g of the gel sample was added to a 15 cm-diameter round glass. A pleasant consistency for topical application is indicated by a spread ability of 5-7cm. The adhesion test involves applying 0.5 grams of gel to a glass plate, then loading it with 300 grams for five minutes. A minimum of 4 seconds is required for optimum adherence. In order for the active ingredient in the gel preparation to be absorbed, the adhesion test is used to measure how long the gel sticks to the contact surface after application.

12 The population of this research was male Wistar rats (*Rattus norvegicus*), 12 weeks old, with a body weight of 150 – 200 grams. The samples utilized in this study were determined using Ferderer's formula, with a 10% correction applied if there were instances of experimental animal mortality during the study period. As a result, the total sample size for this study comprised 15 mice. Prior to the research, the mice underwent a two-week adaptation period, during which their food, drink and maintenance adhered to hospital standards. Additionally, they received worm medicine and vitamins. The extraction procedure commenced with the weighing of all rats, were anesthetized with doses adjusted based on their body weight. Tooth extraction was performed on the left first molar.

The experiment includes three groups: Group A received gel base as a negative control (5 rats), Group B received 2% n-hexane extract gel of red shoot leaves (5 rats), and Group C received 2% ethanol extract gel red shoot leaves (5 rats). For 30 days, each rat in each treatment group received 0.1 cc of gel once day using cotton pellets. The rats were put to death on the 30<sup>th</sup> day by the over-ether inhalation method. The mandibular bone remained intact; the mandibular skin was cleansed, using blade number 11, the mandibular bone was cut from the right to the left temporomandibular joint. The cut was then repeated until only the left mandible remained. After the cut was made, the mandibular bone was cleaned with NaCl until no blood remained. The assessment involves bone remodeling area from radiograph.

A portable veterinary dental X-ray radiography system, the D-X2V, was then used to take pictures of the preserved alveolar bone samples. The radiographic analysis that is calculated is the area of the defect area after tooth extraction on the radiograph. Measurements were carried out using rearrangement measurements on ImageJ software. The procedure involves selecting the radiograph for measurement, zooming in until the image is clear, initiating the measurement with the rectangular feature, recording measurement, and generating the results.





**Image 1.** Mandibular Fixation(A), Mandibular Exposure (B). Bone Density Analyzed (C)





The SPSS 25 statistical data processing software was used to statistically evaluate the results, which included a Shapiro-Wilk test for normality and an evaluation of bone mineral density. Bone mineral density data that has been normal is then subjected to a homogeneity test, which uses the Levene test to homogenize data between groups with a 95% significance level ( $p \geq 0.05$ ). The One-Way Anova parametric test can be used to determine the bone mineral density between groups in data that is normally distributed and homogeneous. After the Anova data revealed a significant difference ( $p < 0.05$ ), the data analysis was carried out to determine the influential group using the Multiple Comparisons Test Type Least Significant Different (LSD).

**RESEARCH FINDINGS**







The results of the Phytochemical test in table 1. show that the n-hexane extract of red shoot leaves contains steroid and triterpenoid compounds, but the results are negative for alkaloid compounds and in table. 2 shows that the ethanol extract of red shoot leaves contains flavonoid, alkaloid and tannin compounds.

**Table 1.** Results of phytochemical tests of n-hexane of red shoot leaves

Secondary Metabolite Compounds	Test Method	Before	After	Test results
Alkaloid	Chlorophom ammoniacal + H2SO4 2N + Dragendorff reagent			(-) no orange brown color

Triterpenoid	Reagent H <sub>2</sub> SO <sub>4</sub> concentrated 2 drops + CH <sub>3</sub> COOH (Acetic acid) Anhydrous 1 drop			(+) dark brown color
Steroid	Reagent H <sub>2</sub> SO <sub>4</sub> concentrated 2 drops + CH <sub>3</sub> COOH (Acetic acid) Anhydrous 1 drop			(+) bluish green color

**Table 2.** Results of Phytochemical Tests of Ethanol Extract of Red Shoot Leaves

Secondary Metabolite Compounds	Test Method	Before	After	Test results
Flavonoid	<i>Chlorophom ammoniacal</i> + <i>Mg powder</i> + <i>alcohol:HCl</i> + <i>amyl alcohol</i>			(+) red color
Alkaloid	Chloroform ammonia White precipitate + H <sub>2</sub> SO <sub>4</sub> 2N + (+) Dragendorff's reagent			(+) white sediment
Tanin	FeCl <sub>3</sub> 1% 2 drops			(+) blackish green color

This study was conducted in three groups, each group was observed after 30 days. The distribution of the data was initially measured. The mean gray value in ImageJ software displays the findings of the bone mineral density (BMD) analysis. According to table 3, the negative control group's (A) BMD result have a mean gray value is 113.79 mm/pixel. The BMD result for the N-Heksan extract of red shoot leaves gel 2% (B) is 149.68 mm/pixel, and ethanol extract of red shoot leaves 2% gels 129.64 mm/pixel.

**Table 3.** Description of data from groups A, B and C.

Treatment Group	Mean gray value	Std. Deviation	Std. Error
Control (-) Group (A)	113.7980	3.94815	1.76567
N-Heksan 2% (B)	149.6800	3.83693	1.71593

Ethanol 2% (C)	129.6400	4.09182	1.82992
Total	131.0393	15.63351	4.03655

The Shapiro-Wilk normality test results (table 4) for all groups indicated  $p > 0.05$ , indicating that the data in each group were normally distributed.

**Table 4.** Tests of Normality.

	Statistic	Shapiro-Wilk	
		df	Sig.
Control (-) Group (A)	.920	5	.531
N-Heksan 2% (B)	.933	5	.616
Ethanol 2% (C)	.967	5	.854

The homogeneity test (table 5) showed  $p > 0.05$ , suggesting that the data variances were homogeneous. Consequently, a statistical test was performed using the One-Way ANOVA test to determine if there were significant differences among the groups.

**Table 5.** Levene's Test for Homogeneity of variance Result

Levene Test	Significance	Probability
	0.999	$P > 0.05$

From the table 6, it is known that the significance value shows a value of less than 0.05, so it can be concluded that there is a significant difference between the Control, 2% N-Hexane and 2% Ethanol. Subsequently, a post hoc test (table 7) was conducted to determine the differences between groups.

**Table 6.** One-Way ANOVA Test Results

One-way Annova	Significance	Probability
	0.000	$P < 0.05$

**Tabel 7.** post-hoc multiple comparisons (LSD) Result

(I) Group	(J) Group	Mean Difference		
		(I-J)	Std. Error	Sig.
Control (-)	N-Heksan 2%	-35.88200*	2.50474	.000
	Ethanol 2%	-15.84200*	2.50474	.000
N-Heksan 2%	Control (-)	35.88200*	2.50474	.000
	Ethanol 2%	20.04000*	2.50474	.000
Ethanol 2%	Control (-)	15.84200*	2.50474	.000
	N-Heksan 2%	-20.04000*	2.50474	.000

2 The results of the Post Hoc test indicated that there was significant difference between the treatment groups.

## DISCUSSION

16 Following tooth extraction, there are multiple stages in the biological process of bone remodeling, including hemostasis, inflammation, proliferation, and remodeling. Granulation tissue will take its place in the socket wound once the bleeding has stopped. The proliferative phase of the bone remodeling process starts when granulation tissue is progressively replaced by a transient connective tissue matrix that is high in collagen fibers.<sup>3</sup> Additionally, collagen is the main protein present in the bone matrix. COL1 is the most Furthermore, the primary protein found in the bone matrix is collagen. The most prevalent kind of collagen protein, COL1, is employed extensively as a bone-preserving agent to promote tissue regeneration in broken bone. Growth factors released by macrophages toward the end of the inflammatory phase trigger the activation of osteoblast cells. By producing proteins that produce new bone, osteoblast cells serve as indicators of alveolar bone healing. These osteoblast cells proceed into the bone remodeling phase after first emerging during the proliferative phase. Apoptosis or osteocyte cell differentiation are two possible outcomes of the maturation process that osteoblast cells go through. Since osteoblasts and osteocytes are indicators of osteogenesis, the number of these cells was calculated. Collagen thickness was determined since it is a measure of the bone remodeling process, and osteoblasts and osteocytes must function in harmony to remodel during osteogenesis.<sup>2,4,6,8,14</sup>

2  
7 In our study, 2% n-hexane extract gel of red shoot leaves had a higher effect on increasing the alveolar bone mineral density of Wistar rats after tooth extraction. The results of the data in table 6 show that there is a significant difference between the treatment groups. The results (Table 7) show that there is a difference in bone density, this is influenced by the difference in the material and concentration of red shoot leaf solvent. The results of bone remodeling after tooth extraction using n-hexane gel of red shoot leaves 2% are more significant, because n-hexane is a non-polar solvent, so it only extracts compounds with low polarity. The results of bone density evaluation using Image-J software revealed that the deposition of newly formed bone trabeculae started from the socket border, growing through the empty socket.

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1 Bone marrow gaps separated the thick, regularly distributed bone trabeculae. With a few more developed lamellar bone appearances, the freshly created bone is mostly composed of woven bone. At five weeks, the newly bone had nearly filled the socket space. Demonstrates that the density rises in the experimental groups, particularly on day 30. After day 28, the medullary canal and trabeculae appear thickened due to the growth of lamellar bone fibers in the socket area. The thickening of trabeculae in this study is in line with bone healing after tooth extraction. Furthermore, this research shows a process soft bone formation that begins in the 4<sup>th</sup> week, as evidenced by a change in the radiographic image from radiolucent to radio intermediate and radiopaque.<sup>1,7</sup>

The findings of the phytochemical analysis of the red shoot leaf n-hexane extract revealed triterpenoid and steroid compounds, which is contrary to earlier research that found secondary metabolites of alkaloid, steroid, and triterpenoid compounds in the n-hexane fraction of red shoot leaves.<sup>15</sup> The phytochemical test in this study did not identify any alkaloids, which may be because ethanol was employed as the solvent for the maceration process and n-hexane was utilized for the fractionation process. Only polar solvents, like ethanol, can dissolve polar molecules, and only non-polar solvents, like n-hexane, will dissolve non-polar compounds. Although alkaloids are typically found in polar solvents, the dissolving process does not take place when n-hexane, a non-polar solvent, is used in the fractionation process. Since steroids are non-polar substances,

they can be found using n-hexane solvent during the fractionation process. By blocking the phospholipase enzyme via the arachidonic acid pathway, steroids can contribute to the inflammatory phase.<sup>10,14,16</sup> It is arachidonic acid that forms prostaglandins. When there is cell injury, prostaglandins are released. The enzymes prostaglandin G and prostaglandin H synthetase, sometimes referred to as cyclooxygenase (COX), mediate the first production of prostaglandins. Arachidonic acid is converted by this enzyme into prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), which is then converted into prostacyclin (PGI<sub>2</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), and thromboxane A<sub>2</sub> (TXA<sub>2</sub>). The eventual outcome will be the appearance of inflammation-related symptoms. By boosting the size and quantity of osteoclasts and inducing the activation of preexisting osteoclasts, prostaglandins actually cause bone resorption. The synthesis of prostaglandins will be inhibited by steroids.<sup>16</sup>

Additionally, by preventing the creation of OLCs (Osteoclast-Like cells), triterpenoids can protect bone density by preventing the formation of RANKL, which prevents RANKL from binding RANK and non-induction of osteoclasts. It has been demonstrated that triterpenoids reduce the expression of genes unique to osteoclasts (such as tartrate-resistance acid phosphatase) during the osteoclast genesis process, which in turn reduces osteoclast differentiation. Red shoot leaves are the natural source of botulinic acid, a pentacyclic triterpenoid molecule with numerous pharmacological and biological characteristics, including anti-inflammatory effects. By decreasing Akt and IκB phosphorylation and PLCγ<sub>2</sub>-Ca<sup>2+</sup> signaling in pathways involved in early osteoclasts genesis and subsequently suppressing c-Fos and NFATc1, botulinic acid effectively inhibits osteoclastogenesis.<sup>17</sup> Numerous biological molecules, such as macrophage/monocyte colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κB ligand (RANK & RANKL), control osteoclast genesis. When RANKL binds to its receptor RAN, it initiates the first osteoclastogenic signaling. In addition to activating the NF-κB, Akt, and mitogen-activated protein kinase (MAPK) signaling pathways, which include p38, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and calcium ion [Ca<sup>2+</sup>] signaled by phospholipase Cγ (PLCγ), the RANK-RANKL complex starts the recruitment of tumor necrosis factor receptor-associated factor 6 (TRAF6). When these pathways are engaged, important transcription factors like nuclear factor of activated T cells c1 (NFATc1) and the Fos proto-oncogene (c-Fos) are induced, which raises the expression of genes unique to osteoclasts.<sup>18</sup>

However, the assessment of bone remodeling after tooth extraction seems to be subject to some limitations. In this research based on the previous investigation, because it considers the time and cost factors. In this study, animal radiography modality was used to assess the bone remodeling process after tooth extraction. This study has limitations, namely that it cannot analyze the increase in the number of trabeculae because changes in the digitalization process reduce quality. For clinical decision making and to complete this study, histopathological analysis is recommended.

## CONCLUSION

The results of this study indicate that the use of solvents with different polarities can affect bone remodeling. The fastest bone remodeling is found at a concentration of 2% n – hexane. According to the results of the phytochemical screening test, the n-hexane extract included triterpenoid and steroids, but the ethanol extract contained flavonoids, tannins, and alkaloids. The study's conclusions show that bone remodeling may be impacted by the use of solvents with different polarities.

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